CHEMICAL CONTROL OF LARVAE OF THE DOG HOOKWORM ANCYLOSTOMA CANINUM (ERCOLANI)

by

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INTRODUCTION

The general importance of the dog hookworm, Ancylostoma caninum (Ercolani, 1859) has been recognized for years. It was with this species that Looss (1898) discovered the skin penetration of the infective larvae as a mode of entrance into the host body. Still greater significance was attached to this parasite when Wells (1931) found that, in feeding, the dog hookworm sucked blood for a considerable period of time after puncturing the intestinal wall and injecting anti-coagulin into the wound. He found that the worm not only filled its intestine with fresh blood, but continued its sucking activity until the blood oozed out through its anus and formed a pool around it. In fact, Wells (1931) found that a single adult worm in the gut of a dog might draw about 0.3 cc of blood in 24 hours.

As is known, other phases of the life cycle of this worm include the passing of thin-shelled eggs in early cleavage stages with other voided material to the moist earth where hatching occurs. In 10 to 12 hours, most of the eggs hatch into rhabditiform larvae (about 0.25 mm in length). The larvae feed and remain in close proximity to their food of bacteria or decaying organic matter in this first stage. McCoy (1929) found that gram negative rod-shaped bacteria were the essential food of the larvae in developing to the infective stage.

These larvae have been observed to grow to almost twice their original length in about 22 hours after hatching. They

then moult by shedding their cuticular covering and enter upon their second stage of larval development, the non-feeding filariform stage in the soil.

Under suitable conditions of temperature and moisture, the larvae in this stage remain at the surface of the upper layer of the soil mostly within the first half inch of the surface. Cort, Ackert, Augustine and Payne (1922) reported on the activities of infective human hookworm larvae, Necator americanus, in the soil.

As long as factors for life remain favorable, the filariform larvae retain their position on the surface of soil particles singly or in polyp-like masses presumably to await contact with their host. But, if, for example, dryness ensued, they migrated back into the soil. The lateral migratory activities of human hookworm larvae were described by Augustine (1922), and the vertical movement by Payne (1922). The burial of larvae at varying depths and their ability to migrate to the surface during periods up to six weeks were studied by Ackert (1923). Larvae lived in the soil up to 58 days.

The life cycle of A. caninum, as here traced, is completed with the infection of the definitive host, the dog. Following skin penetration or oral infection, the usual migratory routes taken by the infective larvae through the tissues of the host have included the lungs and resulted in their eventual establishment in the small intestine. Once in the intestine, the larvae undergo a 3rd and a 4th moult and may reach maturity.

In recent years, this hookworm has been found to be prevalent in this area. Many cases of canine ancylostomiasis have appeared in local animal clinics and hospitals. An incidence of 61.5 per cent of these nematodes in 96 cats from eastern Kansas has been reported by Ackert (1941). In the same area, Ackert and Furunoto (1949) found A. caninum in 109 of 131 cats, or an incidence of 83 per cent.

The recent success of Beaver and Deschamps (1949) in killing Endamoeba histolytica cysts by the application of acetic acid in human stools and the high incidence of carnivore hookworms in this area led to the present investigation.

REVIEW OF LITERATURE

Efforts to control A. caninum have been directed principally toward treatment of the infected host by the administration of an anthelminthic. The removal of many hookworms from an animal has been readily achieved, but to remove all hookworms from a host has been difficult. Other efforts toward control included the development of immunity to this parasite within the hosts, and some chemical treatment of kennel runways and other infested soil.

Oldt, in 1926, as reported by Cort, Grant, Stoll, et al. (1926) found that 25 per cent ammonium sulfate, stone lime, Chili nitrate or gypsum acted lethally on human hookworm larvae in stored night soil in South China.

The delaying effect of sea water on the development of ova and larvae of Necator americanus was observed by Caldwell and Caldwell (1927). The next year, Fischer found that Ancylostoma duodenale larvae in the soil of mine latrines were killed by sodium chloride brine. He, also, reported that a few minutes' treatment with varying amounts of 10 to 20 per cent concentrations of sodium chloride solution acted lethally on ova of Ancylostomidae.

Penso (1933), as reported in Helminthological Abstracts (1933), tested the effects of various artificial fertilizers on the development of the free living stages of Ancylostoma. He found that all such fertilizers retarded the development of eggs and that one per cent or less of calcium cyanamide and ferrous sulphate killed hatched larvae in a few hours. He pointed to the suitability of the usage of ferrous sulphate since it added value to the soil. He also recommended the use of calcium cyanamide for sterilising human feces against hookworms.

The action of calcium cyanamide against the free stages of A. caninum was reported by Rossi (1935). He stated that good results had been obtained by treating infected dog feces with calcium cyanamide in water at the rate of eight grams of cyanamide for each kilogram of manure.

Stevárel and Sorny (1935) and de Giorgi (1936) found that calcium cyanamide was effective in destroying animal hookworm larvae or preventing the hatching of ova. They agreed that a 0.3 per cent solution of calcium cyanamide was lethal to Ancylostoma ova and larvae.

The hope that various solutions of sulphur and chlorine might be used to kill either infective larvae or adult nematode parasites was expressed by LaPage (1936). He had shown that sulphur and chlorine in some chemicals could exert important influences on the sheathing and exsheathment of larvae.

Underwood (1935) has shown that the sprinkling of a saturated solution of sodium chloride in runs where dogs are kept proved satisfactory in protecting dogs from infection with A. caninum. Eradication of these larvae in dog kennels was also studied by McCullough (1936). He found that dilute solutions of lye would rid the kennels of Ancylostoma larvae.

Widespread use of common salt to kill human hookworms in mines was noted by Parnell (1937). The same year, Raevskaja performed numerous viability tests on the eggs of dog hookworm in vitro, and on the ground, using solutions of phenol, with and without sulphur and corrosive sublimate. He reported that the ova were killed on the ground in about three days by copious irrigation with five per cent phenol. The effect of various solutions on helminth eggs in feces was studied by Scott (1937).

Hamel (1939) indicated that 1.9 pounds of sulphur per 100 square feet mixed with top soil resulted in an acid condition of the soil. The sulphur and the soil bacteria produced sulphuric acid which rendered the soil acidic. Experimentally and in the field, sulphuring of the soil has been found to be of assistance in the control of intestinal parasites.

Many other chemicals, in addition to those heretofore

mentioned, have been utilized by various investigators in attempts to find one that was applicable to destroying developing nematode larvae. For a more complete review of the literature on larval strongyle control, the reader is referred to the references of Parnell (1938, 1939), Cameron (1938) and Levine (1943).

MATERIALS AND METHODS

The preliminary steps in this investigation were concerned with the search for cats that were infected with the hookworm, A. caninum. A thorough search revealed no animals that harbored only these nematode parasites. As a result, it was decided to procure two uninfected cats and to attempt to parasitize them with A. caninum larvae.

Two young cats, four or five months old, were obtained. Fecal examination of these felines by smear and zinc sulfate flotations for a week failed to yield any helminth eggs, and it was concluded that the animals were uninfected.

A source of hookworm ova was found in a young female pointer at the Veterinary Clinic. Fecal smears revealed an abundance of A. caninum ova in the two to eight-cell stage.

The stool samples were cultured in order to obtain infective hookworm larvae for the artificial inoculation of the experimental animals. The method of culture employed was a modification of the Ackert (1923) and White (1927) techniques. All the materials used in this experiment, such as, the soil, spatulas, dissecting

pans, etc., were sterilized in an air oven to prevent the introduction of agents which might interfere with the experiment. Dry heat sterilization was effected at a temperature of 350° F. (177° C.) for one hour. The period of exposure was measured from the time the temperature reached 350° F.

At first, dissecting pans 6.5" by 11.0" in size, were used. A layer of cotton approximately 1.5" in depth was moistened with tap water and placed on the bottom of the pans. This was done to insure a continued moist condition. Sandy soil about 0.5" in depth was spread over the moistened cotton. With the aid of a spatula, the sand was overlaid with infested feces to a depth of 1.25". More sandy soil was sprinkled over the feces to just cover it and animal charcoal, in turn, was spread over the entire culture.

Animal charcoal was used to prevent crust formation on the tops of the cultures due to the evaporation of water, and, also for the ease which it afforded in observing larvae that migrated to the soil surface.

The two dissecting pan cultures thus prepared were placed in a laboratory room having a temperature between 80-90° F. They were overlaid with portions of ordinary window screens to prevent contamination by flies and other insects. The pans were placed in a shady corner of the room and observed daily for proper moisture. Care was taken to keep the soil moist but not saturated. The cultures required no further attention.

Large numbers of larvae were seen on the tops of the

cultures about six days after they were prepared. The larvae were isolated from the soil with the aid of the Baermann apparatus as modified by Cort, et al. (1922).

The top layer of the cultures (about 0.5" to 0.75") was scraped off with the aid of a spatula and placed on a double layered cloth gauze in an ordinary tea strainer having a mesh of 30. A funnel with a diameter of four inches served as a receptacle for the strainer. The funnel was attached to a ring stand and had a piece of rubber tubing with a pinch clamp on the other end. Warm water (about 122° F. or 50° C.) was added to the funnel so that it rose about 0.5" above the layer of gauze when the strainer was put in place. The water was allowed to cool to about 104° F. or 40° C. before the strainer was placed in the water.

To facilitate the isolation process, the culture was left in the Baermann apparatus over night. The larvae, which were attracted by the warmth of the water, migrated down through the gauze and strainer into the stem of the funnel. The mesh of the strainer and the double layer of gauze served to prevent, to a large extent, the passage of sand or other debris into the water containing the larvae. On opening the funnel clamp, the larvae passed in the fluid from the funnel into a tube after which they were concentrated by centrifuging.

To induce infections, both cats were orally given approximately 10 cc of the centrifuged solution with a bulb pipette. No attempt was made to ascertain the number of infective larvae that

were introduced since it was desired only to secure infections.

Both of the animals regurgitated five minutes after inoculation. It then was decided to infest the animals' meat-meal with a smaller volume of the centrifuged solution. The larvae-polluted meat also caused the animals to vomit after the intake of a few bites.

Portions of the centrifuged solution were treated with varying amounts of artificial gastric juice and observed under the dissecting microscope (45x) to ascertain if the gastric juice of the stomach would have any perceptible effect on the larvae in the oral feedings. Artificial gastric juice was made as follows: one part of 0.5 per cent commercial pepsin in two parts of 0.6 per cent hydrochloric acid. No perceptible effect was noticed after inspection at intervals over a one-hour period. The room temperature was 85° F. It was concluded that gastric juice had no effect on the larvae. Hence, there was a possibility of larval penetration of the stomach mucosa.

It was decided then to inoculate the animals subcutaneously with larvae by means of a hypodermic needle. A 10 cc syringe with a 22 gauge needle was prepared for inoculation. The cats were not shaved, nor was any alcohol used. Both of the cats were given three subcutaneous injections of varying doses of the centrifuged solution into the side behind the forelegs. No difficulty was encountered as with the oral inoculation.

The animals showed no obvious symptoms from the treatment with the larvae. Smears of the cats' stool samples were made

daily to discern the first appearance of hookworm ova in the feces or the passage of injected larvae. Ova of A. caninum were first observed in the stool smears 15 days after the oral inoculations were given. Daily smear examinations revealed progressive increases in the number of ova seen with the microscope. The number, then, became somewhat constant and, therefore, it was concluded that infections were established in both of the cats.

Series of experiments were devised for applying various chemical compounds to the fecal-soil cultures. Chemical compounds were prepared in 20 per cent concentrations. Stool samples were collected from the animals and examined by the direct smear method to insure the presence of ova prior to performing any tests.

The preliminary and series experiments were prepared according to the method previously outlined with minor variations as follows: Petri dishes were used instead of the dissecting pans because they allowed more accurate manipulation of the materials. The bottom of a clean Petri dish was lined with a layer of cotton about 0.25 inch thick for absorption of water to keep the culture moist. The amount of water was calculated. A layer of sandy soil about 0.5 inch in depth was sprinkled over the moistened cotton. Feces containing ova of A. caninum was spread over the soil layer with the aid of a spatula. Soil was then sprinkled over the fecal layer to cover it. Water from the tap was added with a 10 ml pipette to make the water concen-

tration in the culture 30 cc (taking into account the water used to moisten the cotton, usually 10 cc). The entire culture was covered with animal charcoal as mentioned previously. With the aid of forceps, the Petri dish was placed in a finger bowl having a diameter of 4.5 inches and a height of two inches. The space around the Petri dish was filled with water nearly to the top of the dish to keep the humidity of the culture constant, and to prevent the escape of larvae.

An experimental culture also was prepared. The procedure was the same as that for the control culture outlined above, except for the addition of the chemicals to be tested and the amount of water applied. The experimental cultures had 15 cc of 20 per cent test substances diluted to 30 cc to make up a 10 per cent concentration of the chemicals to be tested, 6 cc of 20 per cent test substances diluted to 24 cc to make up 5 per cent concentrations and 3 cc of 20 per cent test substances diluted to 24 cc to make up 2.5 per cent concentrations. In both the control and experimental cultures, the total fluid content was always 30 cc. It was found in preliminary experiments that this amount of water produced optimal conditions for larval cultures. The water applied to the cotton layer was included in this amount in each culture. The application of water and of the chemical test substances was done with the aid of a 10 cc pipette so that drops of the fluids used would be rather uniformly spread throughout the cultures.

Larvae appeared in these cultures at varying intervals. The

preliminary test cultures required four to six days for the appearance of larvae, while the series experiments required periods ranging from five to nine days. With the appearance of the larvae, use was made of the Baermann apparatus to isolate them from the fecal-soil cultures.

The recovered larvae were counted in two ways. The choice of the method was determined by the numbers of larvae isolated. When the number was comparatively small, the larvae were counted by withdrawing them individually from the solution with the aid of a bulb pipette.

If large numbers of larvae were present, a dilution counting method was employed. This required the use of a 3" by 4" slide and a 1 ml pipette graduated in tenths of a milliliter. The larvae were killed with 10 per cent formalin, pipetted into centrifuge tubes and centrifuged at slow speed for 15 minutes after which the top one-third of the fluid (about 5 cc) was discarded. This served to concentrate the larvae.

A circle with a diameter of approximately 2.5" was made on a 3" by 4" slide with a red marking pencil. Within the large circle along the periphery, five small circles each having a diameter of approximately 0.5" were drawn. These were labeled A,B,C,D and E.

The solution in the centrifuge tube containing the dead larvae was shaken vigorously to obtain an even suspension of larvae. A portion of the solution was drawn at once to the 0.5 ml mark in a 1 ml pipette and quickly very small drops were placed

within the circles A,B, etc. With the entire field in view, counting with a low power lens (45x) in a dissecting microscope was facilitated and repetition reduced to a minimum. The larvae in the volume of the solution put on the slide were counted and the average number per milliliter of solution was determined. This was considered as the dilution factor, and when applied to the total volume of the solution containing the larvae gave the approximate number of larvae present in the entire solution.

The chemical tests on counted larvae required another variation in technique. Formalin was not added to the solutions since it was desired to have living larvae. The larvae were pipetted into centrifuge tubes and the entire sample was centrifuged at slow speed for 10 minutes. The supernatant fluid was bulb-pipetted from the tubes until only an inch of the fluid remained in the tube. This was put on a 2" by 3" slide in drops with the aid of a 1 ml pipette. Four drops usually were placed on a slide. The larvae were counted with the low power of the dissecting microscope. When the counted larvae approximated 100 on the slide, they were washed off the slide with water from a pipette into a Petri dish with layers of moistened cotton and soil, respectively. Care was taken to include the amount of water used to wash the slide in the determination of the total solution applied to the cultures so that the chemical dilution factor would not go awry. More soil was then sprinkled over the culture and a thin layer of animal charcoal was added as in the other test cultures.

Caution was exercised in preventing "dead" larvae from being introduced into the soil. Larvae that did not move were arbitrarily called "dead". These non-motile larvae were removed from the slides with a needle so that possible re-entrance of "dead" larvae did not occur.

Two such larval cultures were prepared for each chemical test. One was a control and the other a test culture. The amount of water in both cases was taken into consideration so that the dilution factor of the chemical was ascertained. After a 24-hour period, the larvae were isolated by the Baermann apparatus, the fluid centrifuged to concentrate the young worms and a count was made to determine their relation to the original number applied to the cultures. The percentages of recovered larvae were computed. Analyses of the percentages of the test and control specimens led to the possible relation of the effect of the various chemicals on the larvae in fecal-soil cultures.

RESULTS

Preliminary Tests

Preliminary tests were performed primarily to determine the choice, concentration and amount of the chemical that was to be stressed in this study on the chemical control of the developing larvae of A. caninum. These results are shown in Tables 1 and 2.

The results of tests of six comparatively weak organic acids and two usual constituents of fertilizers are presented in Table 1. The feces were not weighed nor was any attempt made to count the number of larvae in the controls since these were only preliminary tests. Larvae, however, were counted in the experimental cultures and compared to the large numbers of larvae found in the respective control cultures.

It was found that all the chemicals shown in Table 1 had an inhibitory effect on the developing larvae of the dog and cat hookworm. The chemicals were applied in 10 per cent concentrations in each case. Two tests with acetic acid and one with trichloroacetic acid killed all larvae. A third test with acetic acid showed only three larvae compared with the large numbers in the controls. Oxalic and citric acid also gave quite good preliminary results. The other compounds, such as, lactic acid, tartaric acid, sodium hydrogen phosphate and ammonium sulfate permitted more larvae to survive.

The order of effectiveness of the compounds tested was acetic acid, trichloroacetic acid, oxalic acid, citric acid, lactic acid, tartaric acid, ammonium sulfate and sodium hydrogen phosphate.

The data in Table 2 comprise the results from the second group of preliminary tests on the compounds tested in Table 1, and additional related compounds, such as sodium acetate, calcium lactate, lead acetate, ferric acetate, amyl acetate, sodium chloride and manganese chloride.

Table 1. First preliminary tests for the chemical control of the larvae of the dog and cat hookworm, Ancylostoma caninum, in fecal-soil cultures.

Control cultures			Experimental cultures		
Date culture prepared	Date larvae appeared	Number larvae isolated	Chemicals applied	Chemicals per cent	Number larvae isolated
7/12/40	7/17/40	Large numbers	Acetic acid	10	3
7/13/40	7/18/40	Large numbers	Acetic acid	10	0
7/14/40	7/19/40	Large numbers	Acetic acid	10	0
7/15/40	7/19/40	Large numbers	Trichloro-acetic acid	10	0
7/16/40	7/20/40	Large numbers	Lactic acid	10	18
7/16/40	7/21/40	Large numbers	Oxalic acid	10	5
7/16/40	7/21/40	Large numbers	Citric acid	10	8
7/17/40	7/22/40	Large numbers	Tartaric acid	10	35
7/18/40	7/23/40	Large numbers	Sodium hydrogen phosphate	10	85
7/19/40	7/24/40	Large numbers	Ammonium sulfate	10	71

Table 2. Second preliminary tests for the chemical control of the larvae of the dog and cat hookworm, A. caninum in fecal-soil cultures. (Larvae appeared on tops of cultures in from 4.5 to 6 days.)

Experimental cultures				Control cultures		
Grams fecal sample	Per cent chemical applied	Number larvae isolated	Number larvae per gram feces	Grams fecal sample	Number larvae isolated	Number larvae per gram feces
7.1	Acetic acid 10	10	1.4	6.6	13,139	1989.8
6.2	Acetic acid 5	49	7.9	7.7	14,610	1864.1
6.3	Acetic acid 2.5	209	33.2	7.6	14,123	1859.0
8.3	3-Cl acetic acid 10	53	6.4	8.0	15,064	1994.5
5.6	Lactic acid 10	38	6.8	5.3	11,756	2218.1
6.3	Oxalic acid 10	33	5.2	5.6	12,160	2175.0
6.6	Tartaric acid 10	80	13.3	6.5	12,748	1961.2
7.4	Citric acid 10	74	10.0	7.4	12,902	1754.3
8.4	Sodium acetate 10	136	15.0	8.2	11,880	1450.0
8.4	Calcium lactate 10	3,550	422.6	7.3	10,580	1435.6
9.8	Saline .9	16,216	1,654.7	8.2	14,619	1782.7
8.1	Ammonium sulfate 10	14	1.8	8.5	13,044	1628.7
9.3	Lead acetate 10	596	64.1	7.7	11,678	1516.6
8.5	Ferric acetate 10	1,922	214.4	8.6	13,866	1542.6
9.6	Manganese chloride 10	1,580	164.7	7.5	11,540	1538.7
9.6	Amyl acetate 10	4,672	486.7	8.2	11,546	1773.9
7.5	Sodium hydrogen phosphate 10	32	4.4	9.0	14,903	1863.1

The feces were weighed and the number of larvae per gram of feces was computed in the experimental and control cultures. Interpretation of the numbers of larvae shown in Table 2 revealed that acetic acid in a 10 per cent concentration killed nearly all the larvae in each gram of feces. The control culture showed a recovery of 1089 larvae per gram of the stool specimen. The number of recovered larvae per gram of feces after the application of acetic acid continued to be small as was similarly found in the previous acetic acid tests. A five per cent acetic acid application acted somewhat similarly to the action of a 10 per cent trichloroacetic acid test. Ammonium sulfate and sodium hydrogen phosphate acted quite lethally in 10 per cent concentrations as shown by the numbers of larvae in the experimental and control cultures.

The related compounds of acetic acid proved to be not too effective. The following compounds in 10 per cent concentrations were less effective than a 2.5 per cent concentration of acetic acid: calcium lactate, lead acetate, ferric acetate, amyl acetate, manganese chloride and sodium chloride. Sodium acetate, however, fared comparatively well with 10 per cent concentrations of the other organic acids in its action.

The order of effectiveness of the compounds in 10 per cent concentrations was: acetic acid, ammonium sulfate, sodium hydrogen phosphate, oxalic acid, trichloroacetic acid, lactic acid, citric acid, tartaric acid, sodium acetate, lead acetate, manganese chloride, ferric acetate, calcium lactate and amyl

acetate.

The numbers of larvae recovered from the control cultures varied from 1,435 to 2,219 recovered larvae per gram of feces. Possible factors for these variations will be considered in the discussion.

Effect of Chemicals on Counted Larvae

Table 3 records the effect of six chemicals on counted larvae introduced into soil cultures. The concentrations of the compounds that were employed ranged from 2.5 per cent to 10 per cent. It is seen that acetic acid and lactic acid in 10 per cent concentrations killed all larvae in one test. The controls showed a larval percentage recovery of 91.5 and 70.3, respectively. Acetic acid acted lethally on all but two larvae out of 110 in another test. Eighty-two out of 92 or 89.2 per cent larvae were recovered in the control culture. The 10, 5 and 2.5 per cent concentrations of acetic acid acted more lethally on the larvae than did any of the other compounds in various concentrations. The lethal effects of 10 per cent concentrations followed in this order: acetic acid, lactic acid, trichloroacetic acid, citric acid, oxalic acid and tartaric acid; while five per cent concentrations revealed this order: acetic acid, trichloroacetic acid, citric acid, oxalic acid, lactic acid and tartaric acid; and concentrations of 2.5 per cent gave this order: acetic acid, lactic acid, citric acid, oxalic acid,

Table 3. The effect of various chemical compounds on counted larvae of A. caninum introduced into soil cultures. (Larvae were five days old when put into cultures and were counted at 24 hour intervals after treatment.)

Experimental cultures				Control cultures		
No. larvae put into cultures	Per cent chemicals applied	Number larvae recovered	Per cent larvae recovered	No. larvae put into cultures	Number larvae recovered	Per cent larvae recovered
110	Acetic acid 10	2	1.8	93	82	88.2
124	Acetic acid 10	0	0	94	86	91.5
84	Acetic acid 5	4	4.8	101	90	89.1
98	Acetic acid 2.5	16	16.3	105	98	93.3
119	3-Cl acetic acid 10	20	16.8	128	99	77.3
126	3-Cl acetic acid 5	88	70.4	114	88	78.1
114	3-Cl acetic acid 2.5	102	89.3	120	93	77.5
165	Lactic acid 10	0	0	138	97	70.3
142	Lactic acid 5	118	83.1	149	115	77.2
153	Lactic acid 2.5	127	83.0	157	124	79.0
83	Citric acid 10	65	75.9	90	72	80.0
96	Citric acid 5	76	80.0	87	79	90.8
89	Citric acid 2.5	74	83.1	91	83	91.2
116	Oxalic acid 10	93	80.2	102	91	89.2
108	Oxalic acid 5	89	82.4	115	103	89.6
121	Oxalic acid 2.5	101	83.5	109	98	89.9
98	Tartaric acid 10	87	88.7	105	94	91.2
107	Tartaric acid 5	97	90.7	96	85	88.5
95	Tartaric acid 2.5	82	86.3	111	97	87.4

tartaric acid and trichloroacetic acid. The per cent of larval recovery in the controls ranged from 70 per cent to 94 per cent.

Test on Worm Stages Affected by Acetic Acid

A test was made with acetic acid to ascertain where its lethal action took place. Ten per cent concentrations of acetic acid were employed since that was found to be the most lethal. The cultures were isolated by the Baermann apparatus on the first, second, etc. to the fifth day after the cultures were prepared. Control cultures also were prepared. The results are given in Table 4.

It was found that in the first and second day's isolation by the Baermann apparatus that no larvae were recovered from the experimental cultures. The controls yielded 8 and 54 larvae, respectively. The third day revealed five larvae, the fourth day showed two larvae and the fifth day, 15 larvae from the treated cultures. The number of larvae recovered from the respective untreated cultures was 130, 432 and 14,520.

These results indicated that acetic acid retarded the development of the ova and, also, acted on the larvae that subsequently were hatched.

Series Experiments With Eight Chemicals

The series experiments as depicted in Series 1 to 8 (Tables

Table 4. Results from the application of 10 per cent acetic acid on hookworm larvae, *A. caninum* in cat fecal-soil cultures to obtain information on the stage at which the lethal action occurs. (Larvae were isolated on successive days during a five-day period after cultures were prepared.)

	Experimental cultures			Control cultures		
Isolation days after culture	Grams fecal sample	Number larvae recovered	Larvae per gram of feces	Grams fecal sample	Number larvae recovered	Larvae per gram of feces
1	6.5	0	0	7.6	8	1.1
2	6.8	0	0	6.9	54	7.8
3	6.6	5	0.8	7.2	139	19.3
4	7.0	2	0.3	6.8	482	70.9
5	7.1	15	2.1	6.5	14,520	2235.4

5 to 12) were carried out to check the results that were obtained with the eight most successful chemical compounds tested. Five tests were arranged for each compound, each with a 10, 5 and 2.5 per cent chemical application. Fifteen tests were performed with each chemical compound, in addition to respective controls.

Series 1 (Table 5) shows the results obtained from the application of varying concentrations of acetic acid. The 10 per cent concentrations killed all larvae in two tests and all but 1, 4 and 11 in the other three tests. The average number of larvae recovered per gram of feces in all five tests with 10 per cent acetic acid was less than 0.5, while 1460.9 was the average number recovered in the respective controls. The investigations with five per cent concentrations of the acid revealed an average recovery of 5.3 larvae per gram of feces and an average of 1545.6 larvae in the controls. The 2.5 per cent concentrations, meanwhile, yielded an average recovery of 42.1 larvae and the controls an average of 1460.2 larvae per gram of feces. These results not only confirmed the preliminary ones, but also showed that acetic acid was quite lethal to larvae of A. caninum under the conditions of this investigation.

Trichloroacetic acid was the reagent tested in Series 2 (Table 6). The 10 per cent concentrations of the acid applied to the experimental cultures indicated an average recovery of 7.1 larvae, the five per cent concentrations an average recovery of 16 larvae and the 2.5 per cent concentrations an average recovery of 70.5 larvae per gram of feces. On the other hand,

Table 5 (Series 1). Results from the application of acetic acid on hookworm larvae of A. caninum in cat fecal-soil cultures. (Larvae appeared on tops of cultures from 5.0 to 9.0 days.)

Experimental cultures					Control cultures			
Grams fecal sample	Per cent acetic acid applied	Number larvae isolated	Number larvae per gram feces	Average number per group	Grams fecal sample	Number larvae isolated	Number larvae per gram feces	Average number per group
9.7	10.0	4	0.4		9.2	14,288	1550.2	
11.2	10.0	11	1.0		11.2	16,816	1501.6	
11.8	10.0	0	0		12.2	15,980	1309.9	
10.3	10.0	1	0.1		10.5	16,016	1525.3	
8.2	10.0	0	0		11.2	16,326	1457.7	
				0.3				1468.2
9.0	5.0	41	4.6		10.8	15,988	1480.4	
9.4	5.0	56	6.0		8.8	13,400	1750.0	
9.2	5.0	63	6.8		9.6	14,128	1471.7	
9.8	5.0	43	4.9		9.1	14,250	1566.0	
8.7	5.0	37	4.3		10.8	15,766	1458.8	
				5.3				1545.6
11.1	2.5	426	38.4		9.3	14,002	1505.6	
9.6	2.5	469	50.9		10.4	15,280	1463.5	
8.7	2.5	328	37.7		9.3	14,002	1505.6	
8.5	2.5	302	35.6		10.1	14,962	1481.3	
9.8	2.5	477	47.7		10.1	14,996	1484.0	
				42.1				1488.2

Table 6 (Series 2). Results from the application of trichloroacetic acid on hookworm larvae of A. caninum in cat fecal-soil cultures. (Larvae appeared on tops of cultures in from 5.0 to 9.0 days.)

Experimental cultures					Control cultures			
Grams fecal sample	Per cent trichloro-acetic acid applied	Number larvae isolated	Number larvae per gram feces	Average number per group	Grams fecal sample	Number larvae isolated	Number larvae per gram feces	Average number per group
8.8	10.0	69	7.8		7.9	13,868	1755.4	
8.7	10.0	54	6.2		8.3	13,128	1581.7	
7.8	10.0	53	6.7		8.3	13,008	1550.4	
9.3	10.0	76	8.2		9.0	13,958	1550.9	
8.3	10.0	63	7.6		7.0	13,008	1747.6	
				7.1				1633.2
8.9	5.0	127	14.3		9.1	14,026	1541.3	
9.2	5.0	149	16.3		9.2	14,568	1585.7	
8.2	5.0	126	15.4		7.3	13,672	1572.8	
8.4	5.0	138	16.4		7.7	13,004	1688.8	
8.9	5.0	157	17.6		8.5	14,902	1753.2	
				16				1064.4
8.8	2.5	578	60.2		8.5	14,962	1750.8	
8.6	2.5	682	64.8		8.9	14,336	1610.8	
9.6	2.5	702	73.1		8.2	13,104	1598.0	
7.9	2.5	589	74.6		8.7	13,546	1557.0	
9.1	2.5	725	79.7		8.1	13,014	1606.7	
				70.5				1624.7

the respective controls yielded the following averages of larvae per gram of feces: 1633.2, 1664.4 and 1624.7. Trichloroacetic acid thus, showed promise of a possible application in the control of the dog hookworm.

The third series (Table 7) with sodium hydrogen phosphate disclosed an average recovery of 12.6 larvae in the 10 per cent concentrations, 21.5 larvae in the five per cent concentrations and 200.5 larvae per gram of feces in the 2.5 per cent concentrations. The average numbers of larvae found in the controls were respectively 1629, 1655.4 and 1652.8 larvae per gram of feces. Sodium hydrogen phosphate showed some lethal action on the hookworm larvae, though not as marked as the previous two chemical compounds.

Ammonium sulfate experimental cultures in Series 4 (Table 8) revealed an average recovery of 15.5 larvae in the 10 per cent concentrations, 50.6 larvae in the five per cent concentrations and 208.7 larvae per gram of feces in the 2.5 per cent concentrations employed. The following were the related findings in the controls: 1589.6, 1601.2 and 1592.6 larvae per gram of sample used. The results with ammonium sulfate revealed that this chemical was somewhat similar to sodium hydrogen phosphate in its action on the larvae.

The application of lactic acid as shown in Series 5 (Table 9) displayed an average of 8.8, 20.4 and 94.8 larvae per gram of feces in the 10, 5 and 2.5 per cent experimental cultures, respectively. Meanwhile, the control cultures revealed an

Table 7 (Series 3). Results from the application of sodium hydrogen phosphate on hookworm larvae of A. caninum in cat fecal-soil cultures. (Larvae appeared on tops of cultures in from 5.0 to 9.0 days.)

Experimental cultures					Control cultures			
Grams fecal sample	Per cent sodium hydrogen phosphate applied	Number larvae isolated	Number larvae per gram feces	Average number per group	Grams fecal sample	Number larvae isolated	Number larvae per gram feces	Average number per group
8.2	10.0	89	10.9		8.6	13,124	1526.0	
8.9	10.0	125	14.0		6.8	11,292	1659.1	
7.5	10.0	78	10.4		7.1	12,122	1707.3	
8.1	10.0	102	12.6		7.0	11,330	1638.4	
7.9	10.0	121	15.3		6.8	10,998	1617.3	
				12.6				1629.0
8.2	5.0	195	21.2		8.3	13,206	1591.1	
8.5	5.0	206	24.2		7.9	13,148	1664.3	
8.2	5.0	166	20.2		6.8	11,003	1618.3	
7.9	5.0	155	19.6		7.8	13,016	1669.7	
8.2	5.0	183	22.3		7.5	13,004	1733.9	
				21.5				1655.4
8.7	2.5	1,848	212.4		8.8	14,242	1618.4	
8.3	2.5	1,582	190.6		7.4	12,956	1750.8	
6.9	2.5	968	138.8		6.5	10,546	1622.5	
6.5	2.5	859	132.1		6.3	10,100	1603.2	
8.3	2.5	2,682	323.6		7.8	13,018	1669.0	
				200.5				1652.8

Table 8 (Series 4). Results from the application of ammonium sulfate on hookworm larvae, A. caninum in cat fecal-soil cultures. (Larvae appeared on tops of cultures in from 5.0 to 9.0 days.)

Experimental cultures					Control cultures			
Grams fecal sample	Per cent ammonium sulfate applied	Number larvae isolated	Number larvae per gram feces	Average number per group	Grams fecal sample	Number larvae isolated	Number larvae per gram feces	Average number per group
8.4	10.0	95	11.3		8.1	13,100	1616.0	
6.5	10.0	72	11.1		7.2	11,620	1613.9	
8.0	10.0	121	15.1		6.8	10,013	1473.2	
6.5	10.0	129	19.8		7.4	12,584	1700.5	
7.3	10.0	146	20.0		7.1	10,964	1544.2	
				15.5				1589.6
7.8	5.0	321	41.6		8.6	12,982	1509.5	
8.1	5.0	449	55.4		8.3	12,254	1476.4	
7.3	5.0	289	39.6		6.5	9,982	1532.6	
7.9	5.0	453	57.3		6.8	11,003	1618.8	
6.8	5.0	402	59.1		6.6	12,004	1818.8	
				50.6				1591.2
8.2	2.5	1,899	231.6		8.0	12,904	1613.0	
7.2	2.5	1,528	212.2		7.1	10,532	1490.4	
6.8	2.5	1,252	184.1		6.3	9,548	1515.6	
7.1	2.5	1,672	235.5		6.6	10,906	1650.9	
6.4	2.5	1,154	180.3		6.3	10,666	1693.0	
				208.7				1592.6

Table 9 (Series 5). Results from the application of lactic acid on hookworm larvae of A. caninum in cat fecal-soil cultures. (Larvae appeared on tops of cultures in from 6.0 to 9.0 days.)

Experimental cultures					Control cultures			
Grams fecal sample	Per cent lactic acid applied	Number larvae isolated	Number larvae per gram feces	Average number per group	Grams fecal sample	Number larvae isolated	Number larvae per gram feces	Average number per group
7.2	10.0	42	5.8		6.8	11,342	1667.9	
6.8	10.0	51	7.5		6.2	9,548	1540.0	
7.2	10.0	68	9.4		7.3	12,854	1760.8	
6.8	10.0	72	10.6		6.3	9,904	1564.1	
6.5	10.0	69	10.6		6.4	10,124	1581.9	
				8.8				1626.9
7.5	5.0	181	24.1		7.1	12,886	1772.7	
6.3	5.0	102	16.3		6.4	10,122	1581.6	
6.3	5.0	121	19.2		6.8	11,232	1651.8	
7.2	5.0	156	21.5		7.1	12,236	1736.1	
6.3	5.0	132	20.9		6.8	11,382	1673.8	
				20.4				1683.2
7.0	2.5	651	93.0		6.2	9,988	1611.0	
7.1	2.5	642	90.4		7.0	11,625	1660.4	
6.9	2.5	665	96.4		6.5	10,848	1668.9	
7.1	2.5	638	89.9		6.7	11,114	1658.8	
7.0	2.5	729	104.1		7.1	12,006	1691.0	
				94.8				1669.0

average number of 1626.0, 1663.8 and 1656 recovered larvae per gram of the specimen. Lactic acid, like trichloroacetic acid, showed promise as an agent for the chemical control of A. caninum.

Series 6 (Table 10) indicated the results from the application of oxalic acid. Ten, 5 and 2.5 per cent concentrations yielded an average of 10.1, 27.8 and 119.9 larvae per gram, while the respective controls produced 1644.7, 1675.1 and 1675.3 recovered larvae per gram of feces. Oxalic acid acted like sodium hydrogen phosphate in its lethal action on the larvae of the canine hookworm.

The results with citric acid in Series 7 (Table 11) indicated that the average number of larvae that were recovered in the chemical test cultures were 12.2, 24.5 and 133.4, with the 10, 5 and 2.5 per cent concentrations. The per gram recovery in the controls were 1660.5, 1690.2 and 1627.9 larvae. These results indicated that citric acid killed some, though not all, hookworm larvae.

The final compound tested as illustrated in Series 8 (Table 12) was tartaric acid. Its action on the developing forms of A. caninum showed average recoveries of 13.4, 73.0 and 145.6 larvae per gram, respectively, as compared with averages of 1657.3, 1690.1 and 1678.1 larvae per gram of feces in the controls. Tartaric acid, thus, resembled oxalic and citric acids in its action on canine hookworm larvae.

Table 10 (Series 6). Results from the application of oxalic acid on hookworm larvae of A. caninum in cat fecal-soil cultures. (Larvae appeared on tops of cultures in from 5.0 to 9.0 days.)

Experimental cultures					Control cultures			
Grams fecal sample	Per cent oxalic acid applied	Number larvae isolated	Number larvae per fecal	Average number per group	Grams fecal sample	Number larvae isolated	Number larvae per fecal	Average number per group
6.8	10.0	55	8.1		6.3	10,120	1606.3	
7.1	10.0	72	10.1		6.2	9,852	1585.8	
7.1	10.0	86	12.1		6.4	11,004	1719.4	
6.4	10.0	48	7.5		6.9	11,568	1676.5	
7.1	10.0	89	12.5		6.8	11,122	1635.6	
				10.1				1644.7
6.4	5.0	181	28.3		6.6	10,942	1657.9	
6.7	5.0	239	35.7		6.9	11,214	1625.2	
6.8	5.0	258	37.9		6.6	11,150	1689.4	
6.7	5.0	213	31.8		7.2	12,624	1753.3	
7.3	5.0	402	5.5		6.2	10,128	1640.7	
				27.3				1675.1
6.9	2.5	802	116.2		6.5	11,128	1712.0	
6.3	2.5	654	103.8		6.8	10,958	1696.9	
7.2	2.5	936	130.0		6.9	11,832	1714.8	
6.2	2.5	689	111.1		7.1	12,058	1690.3	
7.9	2.5	956	138.6		6.5	10,754	1654.5	
				119.9				1675.3

Table 11 (Series 7). Results from the application of citric acid on hookworm larvae of A. caninum in cat fecal-soil cultures. (Larvae appeared on tops of cultures in from 5.0 to 9.0 days.)

Experimental cultures					Control cultures			
Grams fecal sample	Per cent citric acid applied	Number larvae isolated	Number larvae per gram feces	Average number per group	Grams fecal sample	Number larvae isolated	Number larvae per gram feces	Average number per group
7.5	10.0	88	12.6		6.8	10,868	1672.0	
6.8	10.0	74	10.9		6.8	9,832	1535.9	
6.5	10.0	79	12.2		6.7	11,122	1660.0	
8.2	10.0	101	12.3		6.8	11,006	1667.6	
6.9	10.0	88	12.8		6.9	11,848	1717.1	
				12.2				1660.5
6.9	5.0	199	29.1		6.8	11,650	1713.2	
6.4	5.0	125	19.5		6.9	11,360	1718.8	
6.1	5.0	102	16.7		6.3	10,232	1624.5	
8.6	5.0	251	29.2		6.8	11,542	1697.4	
8.3	5.0	232	28.0		6.8	11,542	1697.3	
				24.5				1690.2
8.0	2.5	928	116.0		6.7	11,422	1704.3	
7.0	2.5	806	115.1		6.4	10,106	1597.1	
6.6	2.5	749	113.5		6.3	10,146	1610.5	
7.0	2.5	809	115.6		6.0	9,673	1613.0	
6.4	2.5	684	106.9		6.2	10,008	1614.2	
				113.4				1627.9

Table 12 (Series 8). Results from the application of tartaric acid on hookworm larvae of *A. caninum* in cat fecal-soil cultures. (Larvae appeared on tops of cultures in from 5.0 to 9.0 days.)

Experimental cultures					Control cultures			
Grams fecal sample	Per cent tartaric acid applied	Number larvae isolated	Number larvae per gram feces	Average number per group	Grams fecal sample	Number larvae isolated	Number larvae per gram feces	Average number per group
8.1	10.0	109	12.2		8.2	9,946	1604.2	
6.1	10.0	68	11.1		6.1	9,736	1604.6	
6.4	10.0	79	12.3		6.2	10,124	1632.9	
7.5	10.0	101	13.5		7.5	12,348	1691.5	
7.5	10.0	132	17.7		6.8	11,242	1653.2	
				13.4				1637.3
7.9	5.0	258	32.7		7.0	11,982	1708.9	
8.8	5.0	201	23.6		7.1	12,232	1722.8	
6.0	5.0	142	236.7		7.1	12,116	1706.5	
6.9	5.0	209	30.3		8.5	11,144	1714.5	
7.8	5.0	279	35.8		6.4	10,226	1597.8	
				73.0				1690.1
6.3	2.5	848	134.6		6.8	11,546	1684.7	
6.3	2.5	902	143.1		6.8	11,596	1703.8	
6.9	2.5	994	144.1		6.8	11,518	1693.8	
7.2	2.5	1,200	167.8		6.1	10,000	1639.5	
7.2	2.5	998	138.6		7.0	11,682	1668.9	
				145.6				1673.1

General Trends

The results of tests with 15 chemical compounds on the larvae of the dog and cat hookworm, A. caninum in fecal-soil cultures revealed some lethal action in all the tests. The lethal effects of seven of the compounds were so low that no further consideration was given to them. They were: sodium acetate, calcium lactate, sodium chloride, lead acetate, ferric acetate, manganese chloride and amyl acetate.

On the other hand, eight of the compounds used in the preliminary tests appeared to be quite promising. In tests with 10 or 5 per cent concentrations, the lethal action of acetic acid on the larvae was very effective; that of trichloro-acetic acid and lactic acid was noteworthy. Concentrations of 10 or 5 per cent were found to be somewhat less lethal to the larvae in the tests with oxalic acid, citric acid, sodium hydrogen phosphate, tartaric acid and ammonium sulfate.

DISCUSSION

Examination of the results on the comparative effects of the various chemical compounds undertaken in this study revealed that only acetic acid was markedly effective in the control of the developing larvae of A. caninum. The larval survival in the experimental cultures was found to be approximately 98 per cent less than that of the control cultures. These results with

acetic acid are nearly in agreement with those of Levine (1949) who found that 10 per cent and 5 per cent concentrations of acetic acid killed all horse strongyle larvae tested in fecal cultures.[1]

The effects of some of the other chemicals tested in this investigation compared favorably with those found by other workers. The results with ammonium sulfate confirmed those of Oldt (1926), who found the compound to be an active constituent in killing human hookworm larvae. Also, Penso (1933) revealed that certain fertilizers, among them ammonium sulfate and sodium hydrogen phosphate, retarded the development of ova of Ancylostomidae. The results with oxalic acid were in agreement with those of Levine (1949), who found that the compound killed horse strongyle larvae. The other chemicals, as far as was known, have not been tested experimentally for killing hookworm or other strongyle larvae.

While the wide difference between the number of larvae recovered from the experimental cultures and that from the controls is significant, there doubtless were other physico-chemical factors involved besides the action of acetic acid in killing the larvae in the cultures. Nevertheless, these factors would be present in both the experimental and control cultures, and, hence, should not alter markedly the comparative results.

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[1]The findings of Levine came to the writer's attention after the present investigation had been completed.

SUMMARY

An investigation was made of the possible lethal effects of acetic acid and some other chemical compounds on larvae of the dog and cat hookworm, Ancylostoma caninum in fecal-soil cultures.

The sources of hookworm eggs were worms in two young male cats which were artificially inoculated. Larvae were recovered from fecal-soil cultures with the aid of the Baermann apparatus.

In the preliminary tests and the series experiments, the compound most toxic to the larvae of A. caninum was found to be acetic acid since it killed the most larvae in concentrations ranging from 2.5 to 10 per cent.

Results on subjecting counted larvae introduced into soil cultures to the action of the chemicals, demonstrated that all the compounds, acetic acid in particular, acted to some extent upon the larvae.

This investigation revealed that the application of 2.5 to 10 per cent concentrations of acetic acid to fecal-soil cultures in Petri dishes killed about 98 per cent of the larvae of A. caninum treated.

Results of tests with 15 chemical compounds showed some lethal action in all the tests. Apparently, 10 of the compounds were tested for the first time on hookworm or other strongyle larvae. They were: trichloroacetic acid, lactic acid, tartaric acid, citric acid, sodium acetate, calcium lactate, lead acetate,

ferric acetate, manganese chloride and amyl acetate.

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